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(REV 10-96)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

MSK.P-003-USNP

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)

09/214984

INTERNATIONAL APPLICATION NO.

PCT/US97/11982

INTERNATIONAL FILING DATE

16 July 1997

PRIORITY DATE CLAIMED

17 July 1996

TITLE OF INVENTION

Purified Compositions of 10-Propargyl-10-dAM and Method of Using same

APPLICANT(S) FOR DO/EO/US

Sirotnak et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

PCT/US97/11982

MSK P-003-IISNP

17 ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO ..... \$910.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
..... \$700.00No international preliminary examination fee paid to USPTO (37 CFR 1.482)  
but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$770.00Neither international preliminary examination fee (37 CFR 1.482) nor  
international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1040.00International preliminary examination fee paid to USPTO (37 CFR 1.482)  
and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 490.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS

NUMBER FILED

NUMBER EXTRA

RATE

Total claims

8 - 20 =

-16

X \$22.00

\$ 0.00

Independent claims

4 - 3 =

X \$80.00

\$ 78.00

MULTIPLE DEPENDENT CLAIM(S) (if applicable)

+ \$260.00

\$

TOTAL OF ABOVE CALCULATIONS =

\$

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement  
must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

\$

SUBTOTAL =

\$

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

TOTAL NATIONAL FEE =

\$

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be  
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +

\$

TOTAL FEES ENCLOSED =

\$ 568.00

Amount to be:  
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\$

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a. ☒ A check in the amount of \$ 568.00 to cover the above fees is enclosed. check no. 4650b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
overpayment to Deposit Account No. 15-0610. A duplicate copy of this sheet is enclosed.NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR  
1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

Oppedahl & Larson LLP  
P.O. Box 5270  
Frisco, CO 80443

SIGNATURE

Marina T. Larson, Ph.D.  
NAME

32,038

REGISTRATION NUMBER

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**STATEMENT CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION**

Docket Number (Optional)  
**MSKP003USNP**

Applicant, Patentee, or Identifier: **Sirotnak, Francis M., et al.**

Application or Patent No.: **(to be assigned - Nat'l Phase of PCT/US97/11982)**

Filed or Issued: **Herewith**

Title: **Purified Compositions of 10-Propargyl-10-Deazaaminopterin  
and Methods of Using Same in the Treatment of Tumors**

I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF NONPROFIT ORGANIZATION **Southern Research Institute**

ADDRESS OF NONPROFIT ORGANIZATION **2000 Ninth Avenue South  
Birmingham, AL 35255**

**TYPE OF NONPROFIT ORGANIZATION:**

☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION

☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3))

☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_ )  
(CITATION OF STATUTE \_\_\_\_\_ )

☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3))  
IF LOCATED IN THE UNITED STATES OF AMERICA

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STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
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I hereby state that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification filed herewith with title as listed above.  
☐ the application identified above.  
☐ the patent identified above.

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING **Alan H. Dean**

TITLE IN ORGANIZATION OF PERSON SIGNING **Vice President, Commercialization**

ADDRESS OF PERSON SIGNING **2000 Ninth Avenue So., Birmingham, AL 35255**

SIGNATURE **Alan H. Dean** DATE **11/7/99**

**STATEMENT CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION**

Docket Number (Optional)  
MSKP003USNP

Applicant, Patentee, or Identifier: Sirotnak, Francis M., et al.  
Application or Patent No.: (to be assigned - Nat'l Phase of PCT/US97/11982)  
Filed or Issued: Herewith

Title: Purified Compositions of 10-Propargyl-10 Deazaaminopterin  
and Methods of Using Same in the Treatment of Tumors

I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below.

NAME OF NONPROFIT ORGANIZATION Sloan-Kettering Institute for Cancer  
ADDRESS OF NONPROFIT ORGANIZATION 1275 York Avenue Research  
New York, NY 10021

TYPE OF NONPROFIT ORGANIZATION:

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(NAME OF STATE \_\_\_\_\_ )  
(CITATION OF STATUTE \_\_\_\_\_ )

☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3))  
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☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED  
STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_ )  
(CITATION OF STATUTE \_\_\_\_\_ )

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☐ the application identified above.  
☐ the patent identified above.

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Each person, concern, or organization having any rights in the invention is listed below:

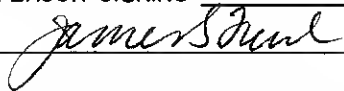
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NAME OF PERSON SIGNING James S. Quirk

TITLE IN ORGANIZATION OF PERSON SIGNING Senior V.P., Research Resources Management

ADDRESS OF PERSON SIGNING 1275 York Avenue, New York, NY 10021

SIGNATURE  DATE 1/11/99

**STATEMENT CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION**

Docket Number (Optional)  
MSKP003USNP

Applicant, Patentee, or Identifier: Sirotnak, Francis M., et al.  
Application or Patent No.: (to be assigned - Nat'l Phase of PCT/US97/11982)  
Filed or Issued: Herewith  
Title: Purified Compositions of 10-Propargyl-10-Deazaaminopterin  
and Methods of Using Same in the Treatment of Tumors  
I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below:  
NAME OF NONPROFIT ORGANIZATION SRI International  
ADDRESS OF NONPROFIT ORGANIZATION 333 Ravenswood Avenue  
Menlo Park, CA 94025

**TYPE OF NONPROFIT ORGANIZATION:**

- ☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
- ☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3))
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(NAME OF STATE \_\_\_\_\_)  
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STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
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I hereby state that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification filed herewith with title as listed above.  
☐ the application identified above.  
☐ the patent identified above.

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Each person, concern, or organization having any rights in the invention is listed below:

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Edward E. Davis  
TITLE IN ORGANIZATION OF PERSON SIGNING Assistant Secretary, SRI International  
ADDRESS OF PERSON SIGNING 333 Ravenswood Avenue; Menlo Park CA 94025  
SIGNATURE [Signature] DATE January 8, 1999

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

03/15/1999 FVDF

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— 65.00 DP

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PURIFIED COMPOSITIONS OF 10-PROPARGYL-10-DEAZAAMINOPTERIN  
AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS

DESCRIPTION

BACKGROUND OF THE INVENTION

This application relates to a purified composition of the compound 10-propargyl-10-deazaaminopterin and to methods of using this compound in the treatment of tumors.

10-Propargyl-10-deazaaminopterin ("10-propargyl-10dAM") is a member of a large class of compounds which have been tested and in some cases found useful in the treatment of tumors. This compound, which has the structure shown in Fig. 1, was disclosed by DeGraw et al., "Synthesis and Antitumor Activity of 10-Propargyl-10-deazaaminopterin," *J. Medical Chem.* 36: 2228-2231 (1993) and shown to act as an inhibitor of growth in the murine L1210 cell line and to a lesser extent of the enzyme dihydrofolate reductase ("DHFR"). In addition, some results were presented for the antitumor properties of the compound using the E0771 murine mammary tumor model. This data was equivocal because of the small number of mice used in the test (3 per dosage), the absence of any standard deviation information which would quantify the reliability of the data, and the fact that the highest dose used was in fact toxic to the mice. Nevertheless, assuming this data has some predictive value for the efficacy of a drug in treating human tumors, it would at best predict a drug which, at equivalent levels of tolerance, had properties comparable to or perhaps slightly better than methotrexate.

SUMMARY OF THE INVENTION

Surprisingly, however, more highly purified 10-propargyl-10dAM compositions when tested in a xenograft model for their efficacy against human tumors have now been shown to be far superior to methotrexate ("MTX") and are even superior to edatrexate ("ETX"), a more recent clinical candidate. Moreover, 10-propargyl-10dAM showed a surprising ability to cure tumors such that there was no evidence of tumor growth several weeks after the cessation of therapy. Thus, a first aspect of the present invention is a highly purified composition containing 10-

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propargyl-10dAM. This composition can be used in accordance with the invention to treat tumors, particularly human mammary tumors and human lung cancer.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the structure of 10-propargyl-10dAM;

Fig. 2 shows an HPLC of an impure 10-propargyl-10dAM preparation prepared in accordance with the prior art;

Fig. 3 shows an HPLC of a highly purified 10-propargyl-10dAM preparation in accordance with the invention;

Fig. 4 shows a synthetic scheme useful in preparing the compound in accordance with the invention;

Fig. 5 summarizes the results of toxicity testing in mice;

Fig. 6 summarizes the results of toxicity testing in rats; and

Fig. 7 shows average plasma concentrations after administration of 10-propargyl-10dAM in dogs.

#### DETAILED DESCRIPTION OF THE INVENTION

This application relates to "highly purified" 10-propargyl-10dAM. As used in the specification and claims hereof, compositions which are "highly purified" contain 10-propargyl-10dAM substantially free of other folic acid derivatives, particularly 10-deazaaminopterin, which can interfere with the antitumor activity of the 10-propargyl-10dAM. A composition within the scope of the invention may include carriers or excipients for formulating the 10-propargyl-10dAM into a suitable dosage unit form for therapeutic use.

10-propargyl-10dAM can be synthesized using the method disclosed in the DeGraw paper, *supra* or in Example 7 of US Patent No. 5,354,751, which is incorporated herein by reference. HPLC evaluation of the product prepared by this method shows the presence of a substantial amount (~4.6%) of an impurity A (Fig. 2) which has a retention time consistent with 10-deazaaminopterin. Thus, if this synthetic approach is employed further purification is necessary beyond that disclosed in the DeGraw et al. paper. Such purification can be carried out by additional HPLC

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or crystallization to remove the 10-deazaaminopterin and other folic acid derivatives which may be present.

Fig. 3 shows an HPLC of a highly purified preparation consisting essentially of 10-propargyl-10dAM in accordance with the invention prepared using the method described in Example 1. In this case, the amount of 10-propargyl-10dAM (as determined by HPLC peak area) approaches 98%, and the peak corresponding to 10-deazaaminopterin is not detected by the processing software although there is a minor baseline ripple in this area.

The highly purified 10-propargyl-10dAM preparation in accordance with the invention was tested for cytotoxicity against human tumor cell lines and antitumor properties using xenografts of human tumor lines in nude mice as described in Example 2. The results of these tests are summarized in Tables 1 and 2. As shown, 10-propargyl-10dAM effected complete regressions of human MX-1 mammary carcinoma to a far greater extent than either MTX (which caused no regressions) or EDX, and was in fact able to effect cures in 9 out of the 20 mice tested. 10-propargyl-10dAM was also far more effective than MTX and EDX against xenografts of human LX-1 lung cancer and led to cures in 4 of the 10 mice tested. Similar results were observed for human A549 lung cancer cells. This level of efficacy is far in excess of anything which could have been predicted based upon the E0771 data which appeared in the DeGraw et al. paper. In fact, in that study no mice treated with the lower, non-toxic dosage level (24 mg/kg) of 10-propargyl-10dAM showed complete regression of the tumors and the average effect of the compound was no better than MTX. These 10-P-dAM treated mice showed an increase in tumor size at the end of three weeks, indicating that a cure had not been effected. It is therefore very surprising that the highly purified compound can be used against human tumors at much lower dosage levels (3 mg/kg) and achieve much higher levels of efficacy and many apparent cures.

While not intending to be bound by any particular mechanism for this increase in activity, it is believed that the presence of even relatively small amounts of other folic acid derivatives such as the 4.6% 10-deazaaminopterin observed in the samples prepared in the DeGraw et al paper can compete with the 10-propargyl-10dAM, effectively inhibiting its activity. This could happen at the level of polyglutamylation of 10-propargyl-10dAM by folyl polyglutamate synthetase in human tumor cells. The



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5 advantage of 10-propargyl-10dAM as substrate for this cytotoxic determinant could be compromised by the presence of 10-dAM which more effectively interacts with this enzyme, but it poorly metabolized thus competitively inhibiting the interaction of 10-propargyl-10dAM with that enzyme. Regardless of the mechanism, however, the highly purified compositions of the invention are markedly more active against human cancer cells than would be predicted based upon the data presented in the DeGraw paper. This is also shown by the increased cytotoxicity of 10-propargyl-10dAM compared to EDX against human tumor cells that was consistently found, and which contrasts with the relative equivalence of these two compounds against murine tumor cells lines as reported by DeGraw et al. This enhanced activity against human tumor cells can be used to provide therapeutic benefits to human patients suffering from cancer, particularly from breast cancer or lung cancer.

10 For this purpose, the highly purified 10-propargyl-10-dAM is advantageously formulated as part of a pharmaceutical preparation. The specific dosage form will depend on the method of administration, but may include tablets, capsules, oral liquids, and injectable solutions for intravenous, intramuscular or intraperitoneal administration. Based upon the relative effectiveness of MTX, EDX and 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin against human xenograft tumors, and on the dosages of MTX and EDX found to be appropriate in human clinical trials, dosages of 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin in the range of from 40 to 120 mg/m<sup>2</sup> of body surface area/day should be effective, depending on the treatment schedule. Higher doses would appear to be contraindicated because of the toxicity observed at such levels in animal studies reported below.

25 10-Propargyl-10-dAM in accordance with the invention may also be formulated in combination with a variety of other cytotoxic and antitumor compounds, including vinca alkaloids such as vinblastine, navelbine and vindesine; 5-fluorouracil; alkylating agents such as cyclophosphamide or ifosfamide; cisplatin or carboplatin; leucovorin; taxols such a paclitaxel or docetaxel; and antibiotics such as doxorubicin and mitomycin. Combinations of 10-propargyl-10dAM with several of these other antitumor agents may also be used.

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EXAMPLE 1

Fig. 4 shows a synthetic scheme useful in preparing 10-propargyl-10-dAM in accordance with the invention. A mixture of 60% NaH in oil dispersion (1.06 g, 26.5 mmol) in 18 mL of sieve-dried THF was cooled to 0°C. The cold mixture was treated with a solution of homoterephthalic acid dimethyl ester (5.0 g, 24 mmol, compound 1 in Fig. 4) in dry THF (7 mL), and the mixture was stirred for 1 hour at 0°C. Propargyl bromide (26.4 mmol) was added, and the mixture was stirred at 0°C for an additional 1 hour, and then at room temperature for 16 hours. The resulting mixture was treated with 2.4 mL of 50% acetic acid and then poured into 240 mL of water. The mixture was extracted with ether (2 X 150 mL). The ether extracts were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to an orange-yellow oil. Chromatography on silica gel (600 mL of 230-400 mesh) with elution by cyclohexane-EtOAc (8:1) gave the product  $\alpha$ -propargylhomoterephthalic acid dimethyl ester (compound 2) as a white solid (4.66) which appeared by TLC (cyclohexane-EtOAc, 3:1) to be homogeneous. Mass spectral data on this product, however, showed it to be a mixture of the desired product 2, and the dipropargylated compound. No starting material 1 was detected. HPLC shows the ratio of mono- to di-propargylated products to be about 3:1. Since the dipropargylated product, unlike compound 1, cannot produce an unwanted coproduct in the next step of the reaction, this material was suitable for conversion to compound 3. Absence of starting compound 1 in the product used to proceed in the synthesis is very important in order to avoid the sequential formation of 10-dAM during the transformations leading to the final product, because complete removal from 10-dAM from 10-propargyl-1-dAM is very difficult.

A mixture was formed by combining 0.36 g of a 60% NaH (9 mmol) in oil dispersion with 10 mL of dry DMF and cooled to 0-5°C. The cold mixture was treated drop-wise with a solution of the product of the first reaction (compound 2) (2.94 g, 12 mmol) in 10 mL dry DMF and then stirred at 0°C for 30 minutes. After cooling to -25 °C, a solution of 2,4-diamino-6-(bromomethyl)pteridine hydrobromide-0.2 2-propanol (1.00 g, 2.9 mmol) in 10 mL dry DMF was added drop-wise while the temperature was maintained near -25 °C. The temperature of the stirred mixture was allowed to rise to -10°C over a period of 2 hours. After an additional 2 hours at -

- 6 -

10°C, the temperature was allowed to rise to 20 °C; stirring at room temperature was continued for 2 hours longer. The reaction was then adjusted to pH 7 by addition of solid CO<sub>2</sub>. After concentration *in vacuo* to remove solvent, the residue was stirred with diethyl ether and the ether insoluble material was collected, washed with water, and dried *in vacuo* to give 1.49 g of a crude product. This crude product was dissolved in CHCl<sub>3</sub>-MeOH (10:1) for application to a silica gel column. Elution by the same solvent system afforded 10-propargyl-10-carbomethoxy-4-deoxy-4-amino-10-deazapteroic acid methyl ester (compound 3) which was homogenous to TLC in 40% yield (485 mg).

A stirred suspension of compound 3 (400 mg, 0.95 mmol) in 2-methoxyethanol (5mL) was treated with water (5mL) and then 10% sodium hydroxide solution (3.9 mL). The mixture was stirred at room temperature for 4 hours, during which time solution occurred. The solution was adjusted to pH 8 with acetic acid and concentrated under high vacuum. The resulting residue was dissolved in 15 mL of water and acidified to pH 5.5-5.8 resulting in formation of a precipitate. The precipitate was collected, washed with water and dried *in vacuo* to recover 340 mg of compound 4 (91% yield). HPLC analysis indicated a product purity of 90%.

Compound 4 (330 mg) was decarboxylated by heating in 15 mL DMSO at 115-120°C for 10 minutes. A test by HPLC after 10 minutes confirmed that the conversion was essentially complete. DMSO was removed by distillation *in vacuo* (bath at 40°C). The residue was stirred with 0.5 N NaOH to give a clear solution. Acidification to pH 5.0 with 1N HCl gave 10-propargyl-4-deoxy-4-amino-10-deazapteroic acid (compound 5) as a yellow solid in 70 % yield. HPLC indicated product purity at this stage as 90%.

Compound 5 (225 mg, 0.65 mmol) was coupled with dimethyl L-glutamate hydrochloride (137 mg, 0.65 mmol) using BOP reagent (benzotriazole-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (287 mg, 0.65 mmol, Aldrich Chemical Co.) in DMF (10 mL) containing triethylamine (148 mg, 1.46 mmol). The mixture was stirred for 3 hours at 20-25 °C and then evaporated to dryness. The residue was stirred with water, and the water-insoluble crude product was collected and dried *in vacuo*. The crude product (350 mg) was purified by silica gel chromatography with elution by CHCl<sub>3</sub>-MeOH (10:1) containing triethylamine

- 7 -

(0.25% by volume) to recover 165 mg of 10-propargyl-10-deazaaminopterin dimethyl ester (compound 6, 50% yield) which was homogeneous to TLC (CHCl<sub>3</sub>-MeOH 5:1).

Compound 6 (165 mg, 0.326 mmol) was suspended in 10 mL stirred MeOH to which 0.72 mL (0.72 meq) 1N NaOH was added. Stirring at room temperature was continued until solution occurred after a few hours. The solution was kept at 20-25°C for 8 hours, then diluted with 10 mL water. Evaporation under reduced pressure removed the methanol, and the concentrated aqueous solution was left at 20-25°C for another 24 hours. HPLC then showed the ester hydrolysis to be complete. The clear aqueous solution was acidified with acetic acid to pH 4.0 to precipitate 10-propargyl-10-deazaaminopterin as a pale yellow solid. The collected, water washed and dried *in vacuo* product weighed 122 mg (79% yield). Assay by elemental analysis, proton NMR and mass spectroscopy were entirely consistent with the assigned structure. HPLC analysis indicated purity of 98% and established the product to be free of 10-deazaaminopterin.

#### EXAMPLE 2

The highly purified 10-propargyl-10dAM preparation prepared in accordance with Example 1 was tested for antitumor properties using xenografts of human tumor lines in nude mice. Xenografts of human MX-1 mammary carcinoma were implanted into nude mice by standard procedures.

To test the antitumor properties of 10-propargyl-10dAM against these tumor cells, 3 mg/kg of the compound was administered once a day to each of twenty mice for a total of five days starting three days after tumor implantation. For comparison, untreated controls (20 mice), methotrexate treated mice (10 mice; dosage 2 mg/kg on the same treatment schedule) and edatrexate treated mice (20 mice; dosage 1.5 mg/kg on the same treatment schedule) were also evaluated. These doses are all "maximum tolerated doses" and thus are an appropriate basis for comparison based upon

equitoxicity. Average tumor diameter was measured 14 days after the start of treatment, i.e., 7 days after the cessation of treatment. Mice which had no measurable tumor at this time were considered to have undergone a complete regression. In addition, mice which were tumor free at 14 days were checked three weeks after cessation of therapy for the reappearance of tumors. Tumor free mice at the end of

- 8 -

three weeks after therapy were considered to be cured. The results are summarized in Table 1.

TABLE 1			
Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	$8.6 \pm 0.9$	0/20	0/20
MTX	$7.6 \pm 0.8$	0/10	0/10
EDX	$2.2 \pm 1.1$	6/20	2/20
10-propargyl-10dAM	0.3	13/20	9/20

As can be seen, 10-propargyl-10dAM is substantially more effective than either MTX or EDX, and effected a substantial number of cures.

#### EXAMPLE 3

Example 2 was repeated using xenografts of human LX-1 lung cancer in nude mice. The results are summarized in Table 2.

TABLE 2			
Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	$10.2 \pm 1.8$	0/10	0/10
MTX	$9.2 \pm 2$	0/10	0/10
EDX	$4.3 \pm 2$	3/10	1/10
10-propargyl-10dAM	0.4	9/10	4/10

Again, 10-propargyl-10dAM was shown to be substantially more effective than MTX or EDX, and effected a substantial number of cures.

#### EXAMPLE 4

Example 2 was repeated using xenografts of human A549 lung cancer in nude mice. The results are summarized in Table 3.

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TABLE 3			
Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	$8.9 \pm 1$	0/5	0/5
MTX	$8.3 \pm 2$	0/5	0/5
EDX	$6.8 \pm 2$	0/5	0/5
10-propargyl-10dAM	$4.2 \pm 2$	3/10	2/10

Again, 10-propargyl-10dAM was shown to be substantially more effective than MTX or EDX, and effected a substantial number of cures.

#### EXAMPLE 5

Cytotoxicity studies were performed on four human tumor cells lines to compare the cytotoxicity of EDX to 10-propargyl-10dAM using a 3 hour pulse-exposure to each compound. Three replicate experiments of each cell line were tested for each compound. The results are summarized in Table 4.

TABLE 4			
Tumor	Tissue Type	IC <sub>50</sub> - EDX	IC <sub>50</sub> 10-propargyl-10dAM
MDA468	lung	$0.38 \pm 0.05$	$0.11 \pm 0.01$
SKLC-16	lung	$0.26 \pm 0.03$	$0.10 \pm 0.014$
ZR-75-1	mammary	$0.86 \pm 0.1$	$0.28 \pm 0.05$
SK-BRIII	mammary	$0.99 \pm 0.15$	$0.14 \pm 0.02$

In each case, the 10-propargyl-10dAM was substantially more cytotoxic than EDX against the human tumor cells lines.

EXAMPLE 6

Toxicity of 10-propargyl-10dAM was assessed in rats, mice and dogs. Male CD rats and male B6D2F<sub>1</sub> mice (Charles River Breeding Laboratories, Wilmington, MA) and young adult male beagle dogs (Marshall Farms USA, Inc., Northrose, NY) were used in the tests. All animals were maintained in environmentally controlled rooms with a 12 hours light/12 hour dark light cycle. Mice and rats were received when 5 weeks old and were observed for 1 to 2 weeks before study and used only if their growth during the preliminary observation matched laboratory standards for weight-gain. Dogs were observed at least 2-3 weeks before use, during which period they were weighted and examined at regular intervals to assure good health. During the test period, all animals were weighted daily and observed for appetite, stool conditions, general appearance and signs of toxicity. Dogs were also examined daily to monitor body temperature, heart rate, and respiration rate.

For all treatments, the dose of drug was weighed and dissolved in isotonic bacteriostatic saline by addition of about 2 molar equivalents of 1N NaOH. The pH of this solution was adjusted to 7-7.2 by addition of NaOH solution as determined using a pH meter. Solutions were used either immediately or after thawing preparations that had been stored at -20°C. Injections in mice and rats were made in a constant volume of 0.01 ml/g of body weight.

Toxicity in Mice

B6D2F<sub>1</sub> mice, five per group, were given 10-propargyl-10dAM i.p. weekly for three weeks (days 1, 8 and 15) at varying concentrations as summarized in Table 5.

Table 5	
Treatment Level	Survivors After 32 days
control	5/5
100 mg/kg	5/5
200 mg/kg	5/5
300 mg/kg	5/5
400 mg/kg	1/5
600 mg/kg	1/5

The results of body weight changes and lethality are summarized in Fig 5. As shown, at 100, 200 and 300 mg/kg there were initial moderate declines in body weight (up to 2 grams), but no further drops in the subsequent doses. All mice in these three dosage groups regained weight in weeks 3 and 4 and survived. At dosages of 400 mg/kg, i.p. QWX3, four out of five mice died on days 19, 20, 23 and 24, and a 600 mg/kg, four out of five mice died on days 9, 18, 19 and 21. Those animals treated with the higher two doses had more than 20% weight loss, ruffled fur and diarrhea. However, surviving mice gained weight and caught up with the control group two weeks after the final injections. The approximate LD<sub>50</sub> was about 370 mg/kg, i.p. QWX3 when estimated with dose effect relationship and the median-effect plot. (Chou et al, *Encyclopedia of Human Biology*, R. Dalbeyco, ed., Vol. 2, pp. 271-279, Academic Press, 1991.)

#### Toxicity in Rats

CD rats, five per group, were given 10-propargyl-10dAM i.v. weekly for three weeks (days 1, 8 and 15) at varying concentrations as summarized in Table 6.

Table 5	
Treatment Level	Survivors After 32 days
control	5/5
50 mg/kg	5/5
100 mg/kg	4/5
150 mg/kg	2/5
200 mg/kg	0/5
300 mg/kg	0/5

The results of body weight changes and lethality are summarized in Fig 6. At 50 mg/kg, i.v. QWX3, no apparent changes in body weight were observed; at 100 mg/kg, there were approximately 10 gram decreases in body weight at the third dose, and one out of the five animals had died by day 23. The remaining animals gained weight, but at a rate less than that of control animals.

At 150 mg/kg, i.v. QWX3, three out of the five rats died on days 18, 20, and 23. At 200 mg/kg, i.v. QWX3 all five rats died on days 12, 14, 15, 20 and 20.



- 12 -

respectively. At 300 mg/kg, i.v. QWX3 all five rats also died, but somewhat sooner than those on the 200 mg/kg dosage, on days 11, 11, 12, 12, and 14, respectively. No immediate toxicity was observed in any of the rats immediately after injection at the 150-300 mg/kg dosages. These rats began to lose weight the following day, and had ruffled fur with evidence of diarrhea and dehydration which culminated one to three days before death. These signs persisted through the course of the experiment and were exacerbated by the second and third injections.

The data from these experiments did not allow a precise calculation of LD<sub>10</sub> or LD<sub>50</sub>. A conservative estimate of LD<sub>10</sub> in rats with the dose-effect relationship and the median-effect plot is about 75 mg/kg, i.v. QWX3 and LD<sub>50</sub> is about 110 mg/kg i.v. QWX3.

#### Toxicity in Dogs

Eight male beagles weighing 9.4 to 10.6 pounds were divided into four pairs. The pairs were treated with intravenous injections of 10-propargyl-10dAM weekly for three weeks (days 1, 8 and 15) at 0 mg/kg (dogs A and B); 3 mg/kg (dogs C and D); 8 mg/kg (dogs E and F) and 12 mg/kg (dogs G and H). At 3 mg/kg and 8 mg/kg, a maximal body weight decrease of 2 to 3 kg occurred on day 20, followed by body weight recovery thereafter through the end of the 35 day observation period. At 12 mg/kg there were steady declines in body weight totaling up to 3 kg (or more than 20% loss), and the animals became moribund on day 12 and 14, prior to the third dosage.

Major signs of toxicity were observed for the dogs treated at 8 mg/kg or 12 mg/kg, including vomiting, diarrhea, watery or bloody stool, lethargy, anoreptic, and generalized weakness. At 3 mg/kg, i.v. QWX3, no symptoms were apparent. The estimated LD<sub>50</sub> is about 8 mg/kg, i.v., QWX3.

Blood samples were drawn from each of the dogs during the testing. No marked or persistent change in blood chemistry or blood cell counts were observed, except at terminal phases of toxicity. There were some declines in white blood cells, lymphocytes, neutrophil counts, decreases in hemoglobin, total protein and albumin, and increases in amylase and monocytes were observed, especially at the higher two doses.

- 13 -

Dogs G and H were euthanized and a complete necropsy was performed on days 12 and 14, respectively. One animal from each of the other pairs were euthanized for histopathological examination on day 33 (dog E) or 34 (dogs B and C) of the experiment.

In Dog G, most of the organs appeared normal. The mucosa of small and large intestines showed edema and hemorrhagic. Stomach and the large and small intestines were empty. Dog H was severely depressed, with shallow breathing and reduced heart rate on day 14 prior to euthanasia. Upon euthanasia, the stomach was found to be filled with bile-tinged mucous, and the intestines were filled with watery stool but no signs of blood. No ulcers in the stomach, intestine or esophagus were observed. The liver was pale and spotty. The spleen was dark purple and rough on the surface.

For dog E which received 8 mg/kg, i.v. QWX3, most organs appeared normal but both sides of the lung were pink with a few bloody spots. Large and small intestines and liver showed lavender color and kidneys showed edema and purple color. Stomach and intestines were full with food, and bladder full with urine.

Dog C, which received 3 mg/kg, i.v. QWX3, appeared normal on day 34 prior to euthanasia. Most organs appeared normal. The right lung was pink, large and small intestines purple in color. Liver showed dark purple color. Stomach and intestines were full with food, and bladder full with urine.

Histopathological examination of tissues of dogs treated with 0 mg/kg and 3 mg/kg i.v., QWX3, showed no significant lesions in the organ specimens collected. At 8 mg/kg, i.v. QWX3, the large intestines and multicoccal mild colitis. At 12 mg/kg, i.v. QWX3, subacute to chronic ulcerative esophagitis and severe necrotizing enterocolitis were observed. Other organs, e.g., brain, heart, liver, lung, kidney, salivary gland, testis and spleen showed no significant lesions.

#### EXAMPLE 7

To monitor the pharmacokinetics of the 10-propargyl-10dAM, single doses of 3 mg/kg were given intravenously to each of two dogs, I and J. Blood samples were collected at -5 min, 5 min, 10 min, 20 min, 30 min, 45 min, 60 min, 90 min, 3 hr, 4 hr, 6 hr, 24 hr, 30 hr and 48 hr. 10-propargyl-10dAM concentrations in

- 14 -

plasma were determined by a fluorometric high performance liquid chromatography (HPLC) method using an Econosphere C18 column, 15% acetonitrile/ $\text{KH}_2\text{PO}_4$  50 mm mobile phase, pH 7.0, with a 1 ml/min flow rate at room temperature. The injection volume was 1  $\mu\text{l}$ . The retention time of 10-propargyl-10dAM was 18.5 minutes.

5 The plasma half-life ( $t_{1/2}$ ) for dog I were 26.7 min, .49 hrs and 37.4 hours for  $\alpha$ ,  $\beta$  and  $\gamma$  phases of the kinetics. For dog J, the observed  $t_{1/2}$  values were 21.2 min, 1.26 hrs and 16.3 hrs. The average plasma concentrations at various times are shown in Fig. 7.

10 Urine specimens were collected from each dog at 30 min, 1 hr, 2 hr and 4 hr following administration of 10-propargyl-10dAM and analyzed by HPLC. 10-propargyl-10dAM was mainly excreted unchanged (retention time 18.5 minutes). There were small amounts of a metabolite with a retention time of 6.3 min which account for <0.31% and <3.5% of the total urinary 10-propargyl-10dAM at 1 hr and 4 hr, respectively.

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CLAIMS

- 1 1. 10-Propargyl-10-deazaaminopterin, substantially free of 10-  
2 deazaaminopterin.
- 1 2. A composition consisting essentially of 10-Propargyl-10-  
2 deazaaminopterin.
- 1 3. A pharmaceutical composition comprising 10-Propargyl-10-  
2 deazaaminopterin, substantially free of 10-deazaaminopterin, and a pharmaceutically  
3 acceptable carrier.
- 1 4. A method for treatment of tumors comprising administering to  
2 a human patient diagnosed as having a tumor a therapeutically effective amount of 10-  
3 propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin.
- 1 5. The method according to claim 4, wherein the tumor is a solid  
2 tumor.
- 1 6. The method according to claim 4, wherein the 10-propargyl-10-  
2 deazaaminopterin, substantially free of 10-deazaaminopterin, is administered in  
3 amounts of from 40 to 120 mg/m<sup>2</sup> of body surface area/day.
- 1 7. The method according to claim 5, wherein the tumor is a  
2 mammary tumor.
- 1 8. The method according to claim 4, wherein the tumor is a lung  
2 tumor.

106 Rec'd PCT/PTO 10 FEB 1998

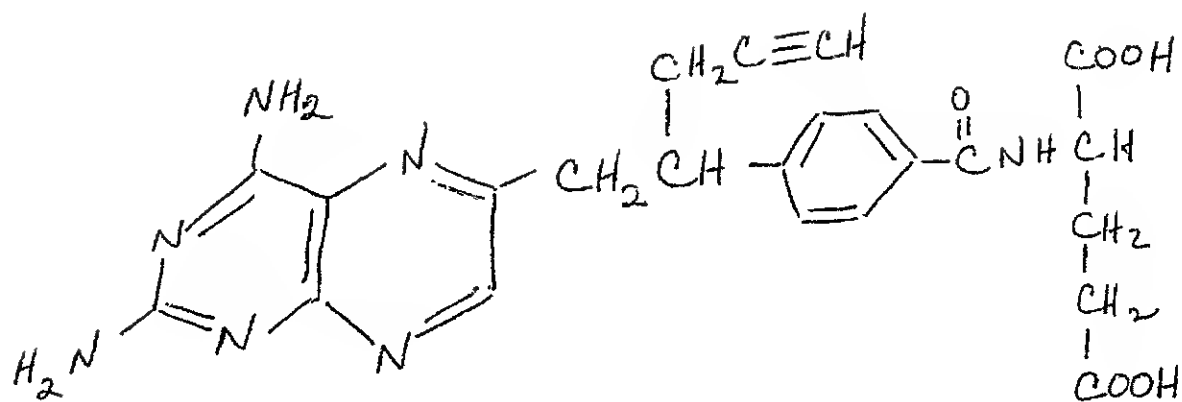
1                   9.     The pharmaceutical composition according to claim 3, further  
2 comprising at least one additional cytotoxic or antitumor compound.

1                   10.    The pharmaceutical composition according to claim 9, wherein  
2 the at least one additional cytotoxic or antitumor compound is selected from the group  
3 consisting of vinca alkaloids, 5-fluorouracil, alkylating agents, cisplatin, carboplatin,  
4 leucovorin, taxols and antibiotics.

1                   11.    The method according to claim 4, wherein at least one  
2 additional cytotoxic or antitumor compound is administered with the therapeutically  
3 effective amount of 10-propargyl-1-deazaaminopterin, substantially free of 10-  
4 deazaaminopterin.

1                   12.    The method according to claim 11, wherein the at least one  
2 additional cytotoxic or antitumor compound is selected from the group consisting of  
3 vinca alkaloids, 5-fluorouracil, alkylating agents, cisplatin, carboplatin, leucovorin,  
4 taxols and antibiotics.

Fig 1



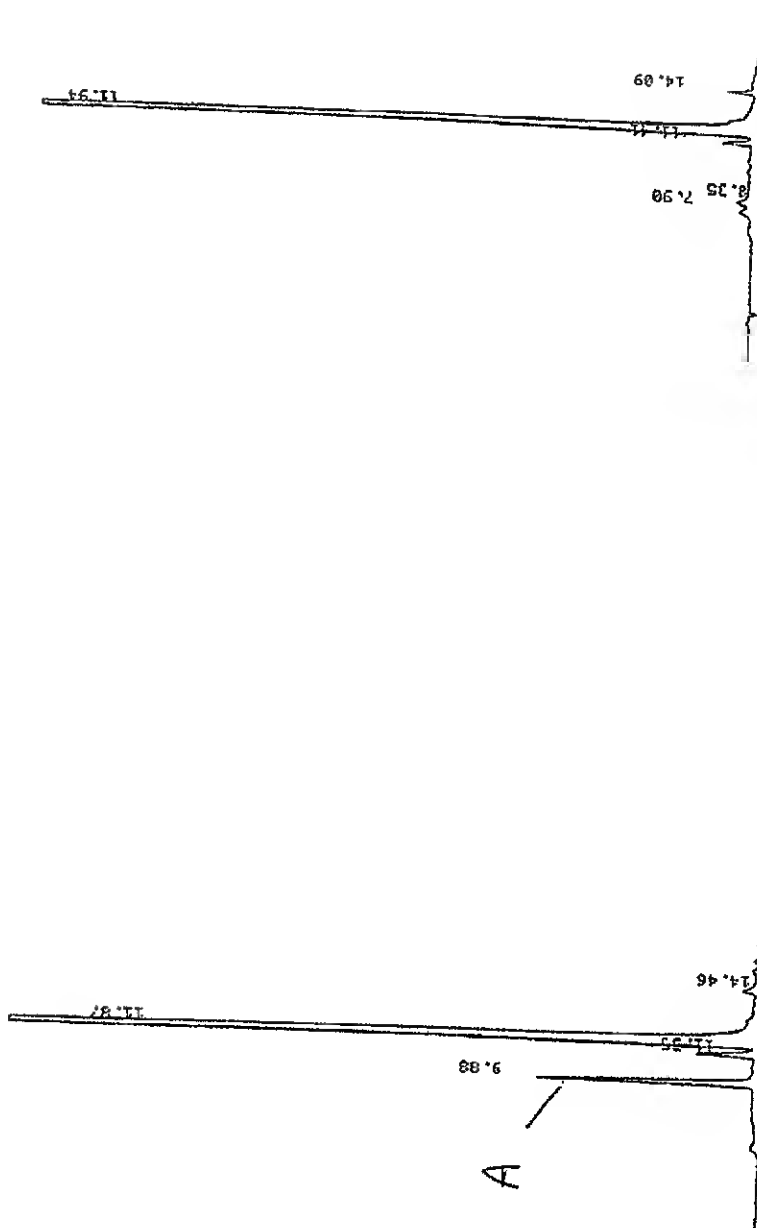
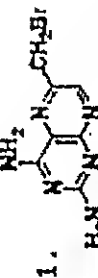
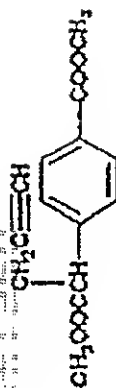


Fig 3

Fig 2

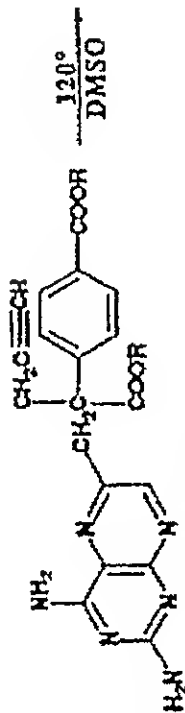
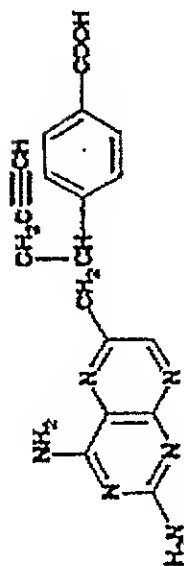


Na H-THF  
Propargyl  
bromide



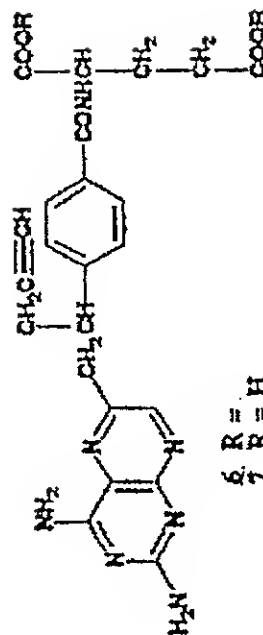
3100-10000

2. 5% NaOH/CH<sub>3</sub>OCH<sub>2</sub>CH<sub>2</sub>OH


$$\begin{array}{c} \text{R} = \text{CH}_3 \\ \text{R} = \text{H} \end{array}$$


42

Me-gluc, BOP,  
Et<sub>3</sub>N, DMF



H  
 II II  
 XX XX  
 5/5

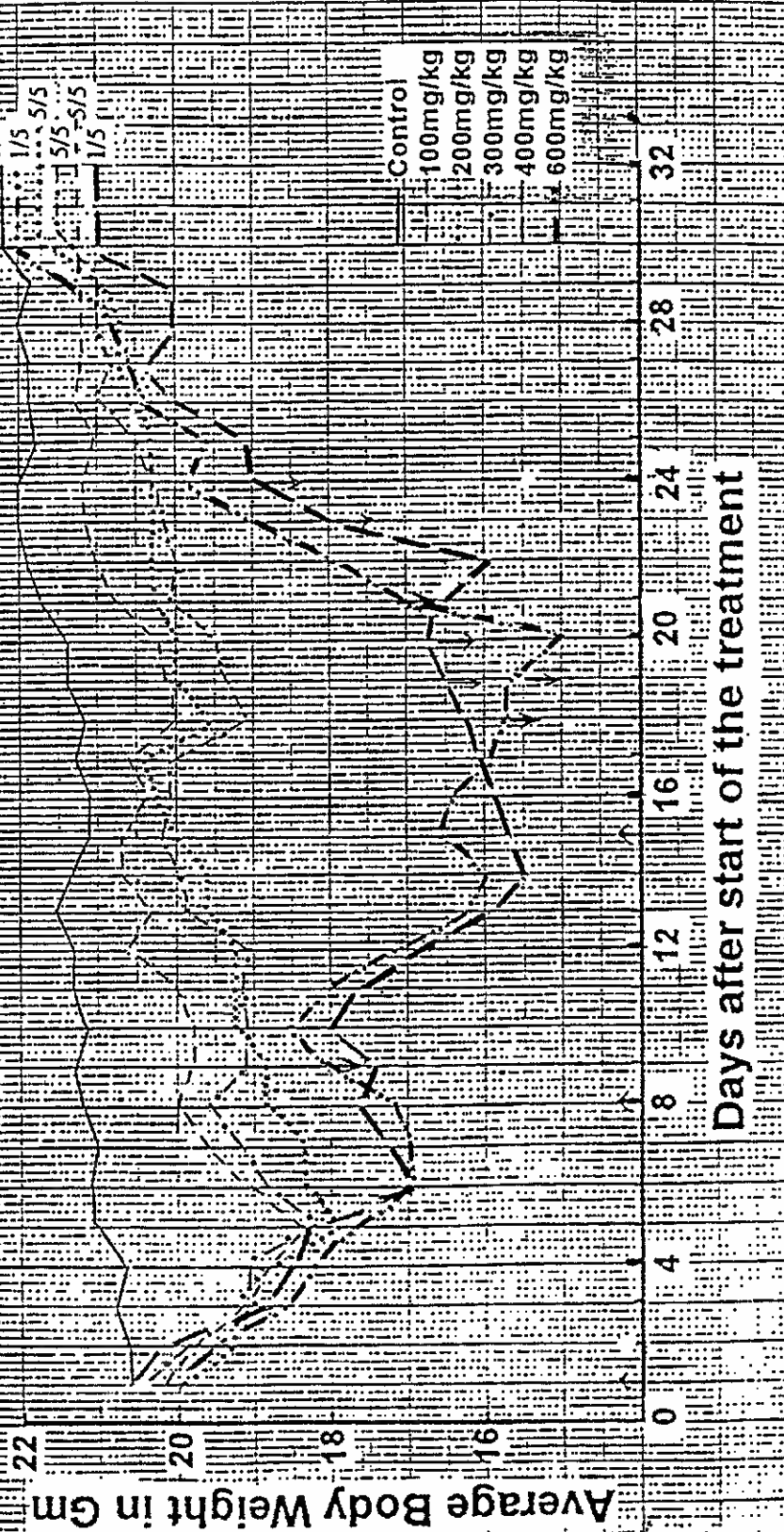
Fig. 4



# FIG 5 TOXICITY OF PDX IN BDF MICE

PDX i.p. QWx3

Five mice in each dose and control  
The arrow signs indicate toxicity death



100, 340, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

NIETZSCH CORPORATION  
MADE IN U.S.A.

# FIG. 6 TOXICITY OF PDX IN CD RATS

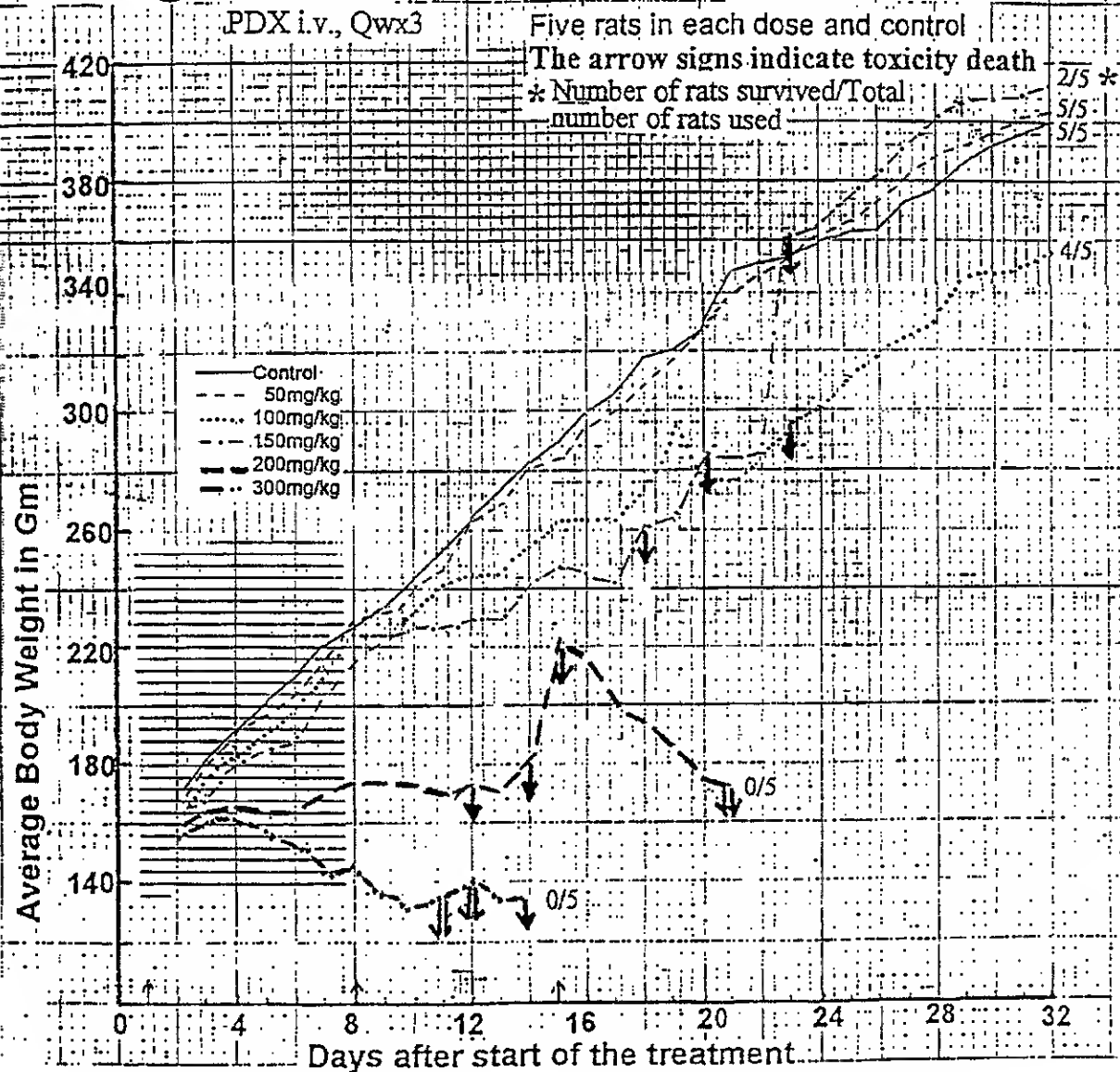
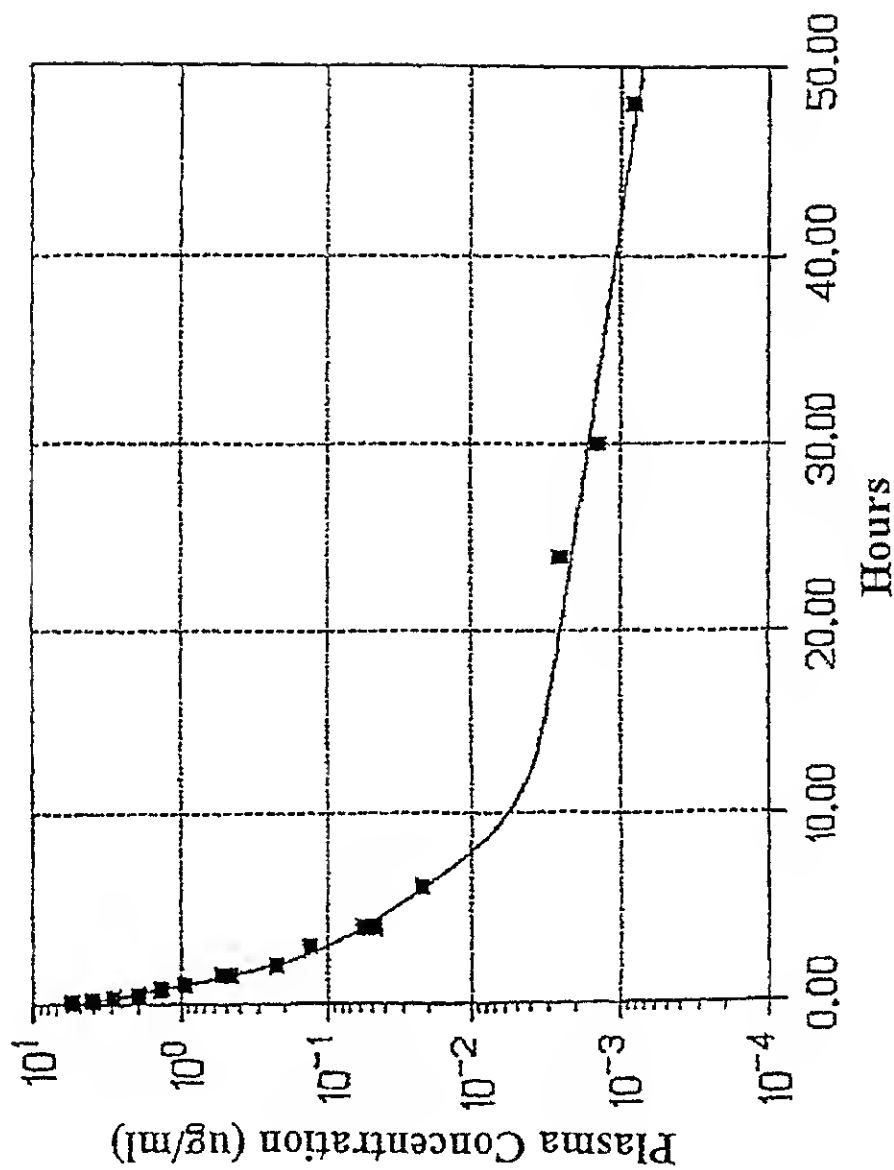


FIG. 7 PLASMA CONCENTRATION OF PDX AT VARIOUS  
TIMES AFTER GIVING 3MG/KG. I.V.,  
A) DOG I & B) DOG J  
B.



## ADDITIONAL INVENTORS

Name of Additional Joint Inventor, if any:

/ / A petition has been filed for this unsigned inventor.

Given Name (first and middle [if any])	:	JAMES R.
Family Name or Surname	:	PIPER
Residence: City	:	Birmingham
State	:	AL
Country	:	US
Citizenship	:	US
Post Office Address	:	P. O. Box 55305
City	:	Birmingham
State	:	AL
Zip	:	35255
Country	:	US

James R. Piper  
(Inventor's Signature)

Date

Name of Additional Joint Inventor, if any:

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Given Name (first and middle [if any])	:	Joseph I.
Family Name or Surname	:	DeGraw
Residence: City	:	Missoula
State	:	MT
Country	:	US
Citizenship	:	US
Post Office Address	:	1255 Snowbowl Drive
City	:	Missoula
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Joseph I. DeGraw  
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Family Name or Surname : COLWELL  
Residence: City : Menlo Park  
State : CA  
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Citizenship : US  
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State : CA  
Zip : 94025  
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\_\_\_\_\_  
William T. Colwell  
(Inventor's Signature)

\_\_\_\_\_  
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#### POWER OF ATTORNEY

8. As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

/X/ Customer Number 021121 Bar Code Label

9. Direct all correspondence to:

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P. O. Box 5270  
Frisco, CO 80443-5270  
Telephone: (970) 668-2050  
Facsimile: (970) 668-2082

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Family Name or Surname : SIROTNAK  
Residence: City : New York  
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Country : US  
Citizenship : US  
Post Office Address : c/o Memorial Sloan Kettering  
Cancer Center  
1275 York Avenue  
City : New York  
State : NY  
Zip : 10021  
Country : US

Date \_\_\_\_\_

Francis M. Sirotnak  
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Post Office Address	:	c/o Memorial Sloan Kettering
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	:	1275 York Avenue
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Francis M. Sirotnak  
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# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

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// Declaration submitted after initial filing (surcharge (37 CFR 1.16(e) required)

As a below named inventor, I hereby declare that:

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## PURIFIED COMPOSITIONS OF 10-PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS

the specification of which  
// is attached hereto  
or

/X/ was filed on (MM/DD/YYYY) \_\_\_\_\_ as United States Application Number or PCT International Application Number **PCT/US97/11982** and was amended on \_\_\_\_\_ (if applicable).

3. I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.
4. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.
5. I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

**Prior Foreign Application Number** :  
**Country** :  
**Foreign Filing Date (MM/DD/YYYY)** :  
**Priority Claimed** :  
**Certified Copy Attached** :

6. I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

**Application Number** : **60/021,908**  
**Filing Date (MM/DD/YYYY)** : **July 17, 1996**

7. I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to

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Attorney Docket No. MSXP003NP

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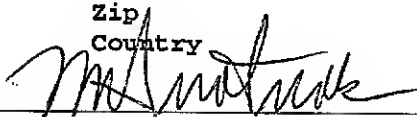
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[illegible]

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James R. Piper  
James R. Piper  
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Date January 7, 1999

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Joseph I. DeGraw  
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Jan 13, 1999  
Date